Biology of Blood and Marrow Transplantation 14:651-657 (2008)

1083-8791/08/1406-0001$32.00/0
doi:10.1016/j.bbmt.2008.03.006

Long-Term Follow-up of Allogeneic Hematopoietic Stem Cell Transplantation for De Novo Acute Myelogenous Leukemia with a Conditioning Regimen of Total Body Irradiation and Granulocyte Colony-Stimulating Factor-Combined High-Dose Cytarabine

Takehiko Mori,[1,2] *Yoshinobu Aisa*,[1] *Reiko Watanabe*,[3] *Rie Yamazaki*,[1] *Jun Kato*,[1,2] *Takayuki Shimizu*,[1] *Naoyuki Shigematsu*,[4] *Atsushi Kubo*,[4] *Tomonaru Yajima*,[5] *Toshifumi Hibi*,[5] *Yasuo Ikeda*,[1] *Shinichiro Okamoto*[1]

[1] Division of Hematology, Department of Medicine, [2] Novartis Pharma Program for Clinical Therapeutics of Hematologic Malignancy, Keio University School of Medicine, Tokyo, Japan; [3] Division of Hematology, Department of Internal Medicine, Saitama Medical Center, Saitama Medical University, Saitama, Japan; [4] Department of Radiology, Keio University School of Medicine, Tokyo, Japan; and [5] Division of Gastroenterology, Department of Medicine, Keio University School of Medicine, Tokyo, Japan

Correspondence and reprint requests: Takehiko Mori, M.D., Ph.D., Division of Hematology, Department of Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan (e-mail: tmori@sc.itc.keio.ac.jp).

Received January 14, 2008; accepted March 18, 2008

ABSTRACT

We retrospectively evaluated the efficacy and safety of total body irradiation (TBI) and granulocyte colony-stimulating factor (G-CSF)-combined high-dose cytarabine as a conditioning regimen for allogeneic hematopoietic stem cell transplantation (HSCT) in patients with de novo acute myelogenous leukemia (AML). The conditioning regimen consisted of 12 Gy of TBI followed by high-dose cytarabine (3 g/m^2) every 12 hours for 4 days in combination with the continuous administration of G-CSF. Stem cell sources included bone marrow or peripheral blood stem cells (PBSC) from human leukocyte antigen (HLA)-identical siblings (n = 24), or bone marrow from HLA serologically matched unrelated donors (n = 26). Fifty patients (median age, 38 years) were evaluated. At HSCT, 35 patients were in the first or second complete remission (CR1/2), and 15 patients were not in remission (n = 14) or in the third CR (n = 1). Thirty-six of 50 patients are currently alive, with a median follow-up period of 5.6 years (range: 1.1-12.1 years). The 5-year estimated overall survival (OS) and disease-free survival (DFS) rates were 85.5% (95% confidence interval [CI], 73.7%-97.3%) and 82.1% (95% CI, 69.0%-95.2%) in patients with AML in the first or second CR, 46.7% (95% CI, 21.4%-72.0%), and 40.0% (95% CI, 15.3%-64.7%) in patients with AML in other stages. The 2-year cumulative incidence of treatment-related mortality (TRM) of all patients was 10.4% (95% CI, 1.8%-18.6%). The only factors affecting the OS and DFS were disease status at transplant and cytogenetics by multivariate analysis. These results suggest that G-CSF-combined high-dose cytarabine could be a promising component of the conditioning regimen for allogeneic HSCT for AML, providing a high DFS and low TRM.

KEY WORDS

Acute myelogenous leukemia • Granulocyte colony-stimulating factor • High-dose cytarabine • Allogeneic hematopoietic stem cell transplantation

INTRODUCTION

Disease recurrence still remains the most important factor interfering with the success of allogeneic hematopoietic stem cell transplantation (HSCT) for acute myelogenous leukemia (AML). Disease-free survival (DFS) of patients with refractory or relapsed

AML after allogeneic HSCT has been reported to range between 21% and 38% [1-11]. Granulocyte colony-stimulating factor (G-CSF) has been shown to increase the susceptibility of some myelogenous leukemia cells to cytarabine in vitro by recruiting quiescent leukemic cells into the cell cycle [12-15]. In this context, several studies have examined the efficacy of the combination of G-CSF with cell cycle-specific chemotherapeutic agents such as cytarabine for refractory myelogenous malignancies [16,17]. Two recent randomized trials have shown that addition of G-CSF to cytarabine-based induction chemotherapy for newly diagnosed AML patients resulted in a significantly higher remission rate or a lower relapse rate [18,19]. In the setting of HSCT, several studies including our previous reports have suggested that G-CSF-combined conditioning for allogeneic HSCT for myeloid malignancies contributed to reduced disease relapse [14,20-23]. To further elucidate the role of this G-CSF-combined regimen in allogeneic HSCT for myelogenous malignancies, we assessed the efficacy of this conditioning with a relatively large number of patients with de novo AML and a long-term follow-up period.

PATIENTS AND METHODS

Patients and Donor Characteristics

This study retrospectively evaluated 50 patients with AML who underwent allogeneic HSCT at Keio University Hospital between January 1995 and April 2006 after being conditioned with total body irradiation (TBI) and G-CSF-combined high-dose cytarabine as described below. Patient and transplant characteristics are shown in Table 1. The diagnosis of AML subtypes was based upon the FAB classification. Patients with therapy-related AML and AML transformed from myelodysplastic syndrome were excluded. Sixteen patients were in the first complete remission (CR1), 19 patients were in the second CR (CR2), 1 patient was in the third CR, and 14 patients were not in CR. Cytogenetic analysis of bone marrow cells was performed by the standard G-banding method. Three cytogenetic risk categories were defined as follows. The favorable-risk category included the abnormalities of inv (16)/t(16;16)/del(16q), or t(15;17) with any additional abnormalities, or t(8;21) without either having del(9q) or being part of a complex karyotype. The intermediate-risk category included +8, −Y, +6, del(12p), and a normal karyotype. The unfavorable-risk category included −5/del(5q), −7/del(7q), inv(3q), abnormalities of 11q, 20q, or 21q, del(9q), t(6;9), t(9;22), abnormalities of 17p, and a complex karyotype. All other abnormalities were included in the intermediate-risk category. Only 1 of the 16 patients in CR1 had a favorable-risk category. Patients were grouped according to the hematopoietic cell transplantation (HCT)-comorbidity index (HCT-ci) assigned at the time of HSCT (scores 0, 1-2, and ≥3) [24-26].

Table 1. *Patient and Transplant Characteristics (n = 50)*

Median age, years (range)	**38 (15-58)**
Sex, Male/Female	**27/23**
FAB subtype	
M0	**4**
M1	**11**
M2	**15**
M3	**8**
M4	**6**
M5	**2**
M6	**1**
Others	**3**
Risk categories of cytogenetics*	
Favorable	**15**
Intermediate	**27**
Unfavorable	**8**
Disease stage at transplant	
First CR	**16**
Second CR	**19**
Others	**15**
Primary induction failure	**3**
Relapse	**11**
Third CR	**1**
HCT-Ci score	
0	**21**
1-2	**15**
≥3	**14**
Stem cell source and donor	
Bone marrow/PBSC from related donor	**19/5**
Bone marrow from unrelated donor	**26**
CMV serostatus	
Recipient + or − / Donor +	**34**
Recipient + / Donor −	**8**
Recipient − / Donor −	**8**
GVHD prophylaxis	
Cyclosporine plus methotrexate†	**22**
Tacrolimus plus methotrexate	**28**

CR indicates complete remission; HCT-ci, hematopoietic cell transplantation-comorbidity index; PBSC, peripheral blood stem cells; GVHD, graft-versus-host disease; CMV, cytomegalovirus.
*Definition was made as described elsewhere [18]. Details are described in the text.
†One patient received cyclosporine alone.

Conditioning Regimen

Twelve Gy of TBI was delivered in 6 fractions of 2 Gy each (3 days) or in 4 fractions of 3 Gy each (2 days). After TBI, cytarabine at a dose of 3 g/m^2 was administered intravenously over 2 hours every 12 hours for 4 consecutive days. Recombinant human G-CSF (lenograstim) was given by continuous infusion at a daily dose of 5 μg/kg, starting 12 hours before the first dose of cytarabine and continuing until the last dose of cytarabine as we have previously reported [20,23]. All patients received steroid eye drops for the prophylaxis of keratoconjunctivitis because of cytarabine. No patients received antithymocyte globulin (ATG) as part of the conditioning regimen.

HSCT Procedure and Supportive Care

Two days after the completion of cytarabine and G-CSF administration, patients received bone marrow transplantation (BMT) or peripheral blood stem cell transplantation (PBSCT). The types of donor and human leukocyte antigen (HLA) compatibility were either HLA-A, -B, or -DR identical sibling, or HLA-A, -B, or -DR serologically matched unrelated donor. T cell depletion of the graft was not performed in any of the patients. For the prophylaxis of graft-versus-host disease (GVHD), patients received cyclosporine A (CSA; 3 mg/kg/day by continuous infusion) or tacrolimus (0.03 mg/kg/day by continuous infusion) with short-term methotrexate (15 mg/m^2 on day 1, and 10 mg/m^2 on days 3 and 6). In cases of unrelated donor marrow transplantation, recipients received additional methotrexate (10 mg/m^2) on day 11. Each patient was isolated in a laminar air-flow room and received prophylactic antibiotics and antifungal agents orally. The administration of lenograstim at a dose of 5 μg/kg was initiated 1 day after HSCT and continued until neutrophil recovery was achieved. Regimen-related toxicities were assessed and graded according to the National Cancer Institute Common Toxicity Criteria (version 2.0).

Engraftment and GVHD

The day of myelogenous engfratment was defined as the first day of 3 consecutive days when the absolute neutrophil counts exceeded 0.5×10^9/L. Graft failure was defined as the lack of myelogenous engraftment in patients surviving in remission for at least 28 days after transplantation. Both acute GVHD (aGVHD) and chronic GVHD (cGVHD) were diagnosed and graded based on the published criteria [27,28].

Statistical Analysis

OS, DFS, relapse, and nonrelapse mortality (NRM) curves were calculated by the Kaplan-Meier method, and comparisons were made using the log-rank test. Variables analyzed were patient age and sex, sex match and mismatch between patient and donor, type of donor, disease status at transplant, risk categories of cytogenetics, cytomegalovirus (CMV) serostatus before transplant, HCT-ci scores, GVHD prophylaxis, development of aGVHD, and any type of cGVHD. For multivariate analysis, we used the Cox proportional hazards model with stepwise regression method. A level of $P < .05$ was considered to be statistically significant.

RESULTS

Regimen-Related Toxicities and Engraftment

Regimen-related toxicities are summarized in Table 2. The regimens were generally well tolerated.

Table 2. *Regimen-Related Toxicities (n = 50)*

	Grades*				
	0	1	2	3	4
Stomatitis	1	4	17	28	0
Diarrhea	0	7	17	26	0
Hepatotoxicity	8	20	16	5	1
Conjunctivitis/Keratit	18	5	5	22	—

*Grades were evaluated accoding to the National Cancer Institute Common Toxicity Criteria (ver. 2.0).

Among the 50 patients, grades 3-4 toxicities were observed for mucositis (n = 28), diarrhea (n = 26), hepatotoxicity (n = 6), and conjunctivitis/keratitis (n = 22). No other grade 3-4 toxicities were observed. There were 3 regimen-related deaths early after HSCT; 2 were caused by bacterial infection, and the other was a primary graft failure. In the remaining 47 patients, myeloid engraftment was achieved at a median of 19 (range: 13-25) days after transplantation.

aGVHD and cGVHD

The cumulative incidences of grades II-IV and III-IV aGVHD were 46.2% (95% confidence interval [CI], 31.9%-60.5%) and 8.2% (95% CI, 0.6%-15.8%), respectively. No patient developed grade IV aGVHD. Only 4 recipients of unrelated donor bone marrow developed grade III aGVHD (15.4%), whereas none of the recipients of sibling donor bone marrow or peripheral blood stem cells developed grade III aGVHD. The cumulative incidences of grades II-IV aGVHD were 34.8% (95% CI, 15.4%-54.2%) among HSCT recipients from sibling donors, and 55.9% (95% CI, 36.5%-75.3%) among HSCT recipients from unrelated donors ($P = .253$). Thirty (68.1%) of 45 evaluable patients developed any grade of cGVHD (extensive-type 27, limited-type 3). The cumulative incidence of any grade of cGVHD was 82.2% (95% CI, 75.6%-88.8%) among HSCT recipients from unrelated donors, which was significantly higher than that among HSCT recipients from sibling donors (51.5%; 95% CI, 29.9%-73.1%, $P < .05$).

Treatment-Related Mortality (TRM), Relapse, and Survival

The 2-year cumulative incidence of TRM was 10.4% (95% CI, 1.8%-18.6%). It was 8.8% (95% CI, 0%-18.2%) in patients with AML in CR1/2 and 14.4% (95% CI, 0%-33.0%) in patients with AML in other advanced stages; these results are not significantly different ($P = .526$; Figure 1A). The causes of TRM were bacterial infection (n = 2), fungal infection (n = 1), aGVHD of grade III (n = 1), and primary graft failure (n = 1). Eleven patients relapsed at 1.3-68.1 months (median 5.9 months) after transplantation. The 5-year cumulative incidences of relapse were 15.6% (95% CI,

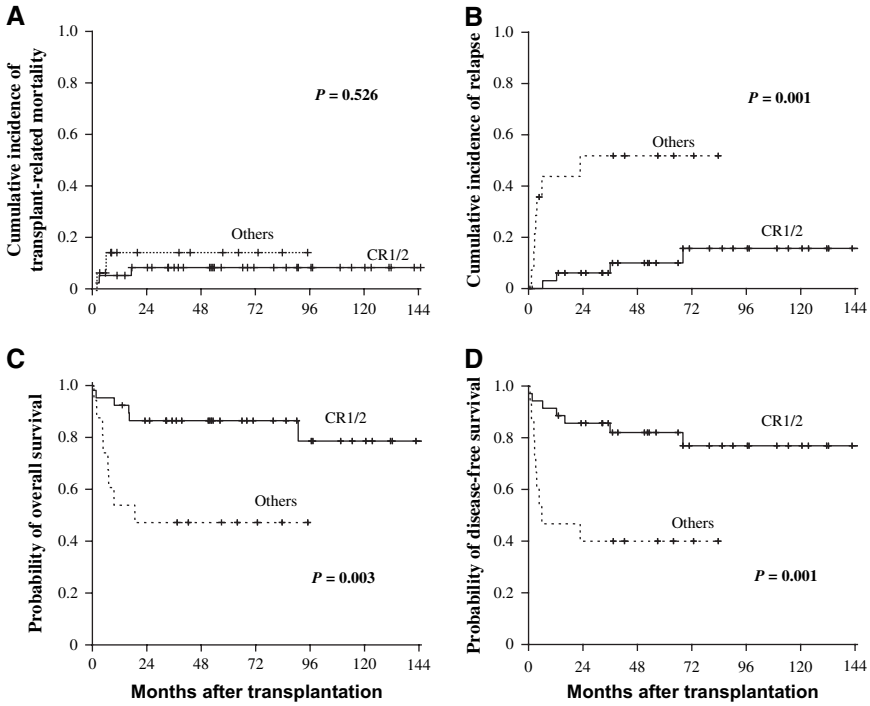

Figure 1. Kaplan-Meier estimates of (A) TRM, (B) relapse rate, (C) OS rate, and (D) DFS rate. The + indicates a censored patient.

9.0%-30.3%) in patients with AML in CR1/2 and 51.8% (95% CI, 24.8%-78.8%) in patients with AML in other advanced stages (P = .001, Figure 1B). The site of relapse was exclusively the bone marrow.

The follow-up period of the 36 patients who were alive at the date of analysis ranged from 1.1 to 12.1 years, with a median of 5.6 years. The 5-year estimated OS was 85.5% (95% CI, 73.7%-97.3%) and 46.7% (95% CI, 21.4%-72.0%) in patients with AML in CR1/2 and in other advanced stages (P = .003, Figure 1C). The 5-year estimated DFS rates were 82.1% (95% CI, 69.0%-95.2%) and 40.0% (95% CI, 15.3%-64.7%) in patients with AML in CR1/2 and in other advanced stages (P = .001, Figure 1D). DFS was not significantly different between patients in CR1 (74.0%, 95% CI 52.0-96.0%; n = 16) and CR2 (88.4%, 95% CI 73.2%-100%; n = 19, P = .115). The 34 patients who are disease-free and alive are all in good condition, and have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 (n = 32) or 1 (n = 2).

Factors Affecting the Survival after Allogeneic HSCT

In univariate analysis, disease status at transplant, risk categories of cytogenetics, aGVHD, and GVHD prophylaxis significantly affected overall survival (OS) (Table 3). Disease status at transplant, patient age at transplant, and aGVHD significantly affected DFS (Table 3). Other factors, including sex, sex match or mismatch between patient and donor, type of donor (related or unrelated), donor age, CMV serostatus before transplant, HCT-ci scores, and any type of cGVHD did not significantly affect the OS and DFS. In multivariate analysis, disease status at transplant and risk categories of cytogenetics significantly affected both OS and DFS (Table 4).

DISCUSSION

Results of this study indicate that TBI and G-CSF combined high-dose cytarabine offers promise as a conditioning regimen for patients with de novo AML undergoing allogeneic HSCT by providing a high DFS rate with a low TRM. In patients with AML in CR1/2 in our study, the cumulative incidence of disease relapse was only 15.6%, which is apparently lower than the reported incidence, as described previously [1-5]. Furthermore, in patients with refractory or relapsed AML, the cumulative incidence of disease relapse was 51.8%, which was lower than the most of the reported rates [7-11]. The combination of G-CSF with chemotherapy is based on the hypothesis that it increases the susceptibility of myelogenous leukemia cells, thereby contributing to a decrease in the relapse rate and further increasing the survival rate [12-19]. Although not evaluated in randomized trials in the setting of HSCT, the results of the previous studies and the present study suggest that G-CSF-combined conditioning for myelogenous malignancies reduces the relapse rate, and further increases the survival rate without increasing TRM [14,20-23]. Because the

Table 3. *Univariate Analysis for Factors Affecting Survival*

	Overall Survival (SE)	Disease-Free Survival (SE)
Disease status at transplant		
CR1 or CR2	0.86 (0.06)	0.82 (0.07)
Others	0.46 (0.13)	0.40 (0.13)
P-value	.003	.001
Sex		
Male	0.77 (0.08)	0.73 (0.09)
Female	0.70 (0.10)	0.65 (0.10)
P-value	.759	.741
Age at transplant		
<40	0.67 (0.09)	0.59 (0.10)
= or >40	0.82 (0.08)	0.82 (0.08)
P-value	.150	.049
Risk categories of cytogenetics*		
Favorable and intermediate	0.78 (0.06)	0.73 (0.07)
Unfavorable	0.50 (0.18)	0.38 (0.17)
P-value	.015	.056
Grades of acute GVHD		
0-I	0.92 (0.06)	0.88 (0.07)
II-IV	0.64 (0.10)	0.58 (0.11)
P-value	.032	.047
GVHD prophylaxis		
CsA-based	0.86 (0.08)	0.81 (0.09)
Tacrolimus-based	0.68 (0.09)	0.63 (0.09)
P-value	.049	.083

SE indicates standard error; CR, complete remission; GVHD, graft-versus-host disease; CsA, cyclosporine A.

*Definition was made as described elsewhere [18]. Details are described in the text.

Table 4. *Multivariate Analysis for Factors Affecting Survival*

	Hazard Ratio (95% CI)	*P*-Values
Overall survival		
Disease status at transplant		
CR1 or CR2	1	.004
Others	5.333 (4.206-6.460)	
Risk categories of cytogenetics*		
Favorable and intermediate	1	.01
Unfavorable	2.157 (1.575-2.739)	
Acute GVHD		
0-I	—	NS
2-4	—	
GVHD prophylaxis		
CsA-based	—	NS
Tacrolimus-based	—	NS
Disease-free survival		
Disease status at transplant		
CR1 or CR2	1	.002
Others	5.284 (4.201-6.325)	
Risk categories of cytogenetics*		
Favorable and intermediate	1	.026
Unfavorable	1.871 (1.318-2.424)	
Age at transplant		
<40	—	NS
> or = 40	—	
Acute GVHD		
0-I	—	NS
2-4	—	

CR indicates complete remission; GVHD, graft-versus-host disease; CsA, cyclosporine A.

*Definition was made as described elsewhere [18]. Details are described in the text.

previous studies of conditioning regimens consisting of TBI and high-dose cytarabine without G-CSF in AML patients is limited, it is also difficult to compare the results of the present study with those of the previous studies to evaluate the effect of combination of G-CSF with conditioning [29,30].

In addition to a low incidence of disease relapse, a notably low incidence of TRM, 10.4%, contributed to a high DFS rate in our study. Incidence of TRM has been reported to range between 17.3% and 49.0% in patients with AML in CR1 or CR2, and between 40% and 45% in patients with refractory or relapsed AML. One possible explanation for this lower TRM rate in our study compared with those reported studies is the small number of cases of serious regimen-related toxicities, resulting in the absence of cases of fatal organ damage. This reduced regimen-related toxicity might result from the absence of cyclophosphamide in our conditioning, which is generally included in most of the myeloablative regimens. Another explanation for the low incidence of TRM is the use of tacrolimus for the prophylaxis of GVHD, mainly for HSCT from an unrelated donor, which might have contributed to the low incidence of severe aGVHD (grade III, 8.2%) without development of grade IV aGVHD. This latter explanation is supported by the result that HSCT from an unrelated donor was not a factor negatively affecting the survival rate in this study, whereas it was so in other studies [11,31].

Because the numbers of cases of relapse and TRM in our study were small, a statistical evaluation of the factors affecting the relapse rate and TRM was not performed. Instead, risk factor analysis was performed for OS and DFS. Several factors, including disease status at transplant, categories of cytogenetic analysis, development of grades II-IV aGVHD, and GVHD prophylaxis, affected OS and/or DFS in a univariate analysis. However, only disease status at transplant and categories of cytogenetic analysis were identified as factors affecting both OS and DFS in a multivariate analysis, which is consistent with the results of reported studies [11,32-34]. Although a G-CSF-combined regimen could have some efficacy in reducing the relapse rate, further improvements are still required for patients with AML in advanced disease status or with unfavorable risk categories of cytogenetic analysis.

In conclusion, TBI and G-CSF-combined high-dose cytarabine might be a promising conditioning regimen for allogeneic HSCT for patients with AML by providing a high survival rate with a low TRM. Further studies to evaluate the efficacy of G-CSF-combined conditioning should be performed in a randomized trial.

REFERENCES

1. Ringdén O, Horowitz MM, Sondel P, et al. Methotrexate, cyclosporine, or both to prevent graft-versus-host disease after HLA-identical sibling bone marrow transplants for early leukemia? *Blood*. 1993;81:1094-1101.
2. Zittoun RA, Mandelli F, Willemze R, et al. Autologous or allogeneic bone marrow transplantation compared with intensive chemotherapy in acute myelogenous leukemia. European Organization for Research and Treatment of Cancer (EORTC) and the Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto (GIMEMA) Leukemia Cooperative Groups. *N Engl J Med*. 1995;332:217-223.
3. Gale RP, Buchner T, Zhang M-J, et al. HLA-identical sibling bone marrow transplants vs chemotherapy for acute myelogenous leukemia in first remission. *Leukemia*. 1996;10:1687-1691.
4. Mehta J, Powles R, Treleaven J, et al. Long-term follow-up of patients undergoing allogeneic bone marrow transplantation for acute myeloid leukemia in first complete remission after cyclophosphamide-total body irradiation and cyclosporine. *Bone Marrow Transplant*. 1996;18:741-746.
5. Litzow MR, Pérez WS, Klein JP, et al. Comparison of outcome following allogeneic bone marrow transplantation with cyclophosphamide-total body irradiation versus busulphan-cyclophosphamide conditioning regimens for acute myelogenous leukaemia in first remission. *Br J Haematol*. 2002;119:1115-1124.
6. Frassoni F, Labopin M, Gluckman E, et al. Are patients with acute leukaemia, alive and well 2 years post bone marrow transplantation cured? A European survey. Acute Leukaemia Working Party of the European Group for Bone Marrow Transplantation (EBMT). *Leukemia*. 1994;8:924-928.
7. Copelan EA, Biggs JC, Thompson JM, et al. Treatment for acute myelocytic leukemia with allogeneic bone marrow transplantation following preparation with BuCy2. *Blood*. 1991;78: 838-843.
8. Michallet M, Thomas X, Vernant JP, et al. Long-term outcome after allogeneic hematopoietic stem cell transplantation for advanced stage acute myeloblastic leukemia: a retrospective study of 379 patients reported to the Société Française de Greffe de Moelle (SFGM). *Bone Marrow Transplant*. 2000;26:1157-1163.
9. Biggs JC, Horowitz MM, Gale RP, et al. Bone marrow transplants may cure patients with acute leukemia never achieving remission with chemotherapy. *Blood*. 1992;80:1090-1093.
10. Clift RA, Buckner CD, Appelbaum FR, et al. Allogeneic marrow transplantation during untreated first relapse of acute myeloid leukemia. *J Clin Oncol*. 1992;10:1723-1729.
11. Fung HC, Stein A, Slovak ML, et al. A long-term follow-up report on allogeneic stem cell transplantation for patients with primary refractory acute myelogenous leukemia: impact of cytogenetic characteristics on transplantation outcome. *Biol Blood Marrow Transplant*. 2003;9:766-771.
12. Miyauchi J, Kelleher CA, Wang C, Minkin S, McCuloch EA. Growth factors influence the sensitivity of leukemic stem cells to cytosine arabinoside in culture. *Blood*. 1989;73:1272-1278.
13. Waga K, Furusawa S, Nagashima S, Saito K, Shishido H. Comparative effects of G-CSF, GM-CSF, and IL-3 on cytosine arabinoside- and daunorubicin-mediated cytotoxicity of acute myeloid leukemia cells and normal myeloid progenitors. *Int J Hematol*. 1992;56:17-27.
14. Takahashi S, Okamoto S-I, Shirafuji N, et al. Recombinant human glycosylated granulocyte colony-stimulating factor (rhG-CSF)-combined regimen for allogeneic bone marrow transplantation in refractory acute myeloid leukemia. *Bone Marrow Transplant*. 1994;13:239-245.
15. Tafuri A, De Felice L, Petrucci MT, et al. Multidrug resistance expression and proliferative studies in poor risk acute myeloid leukemia treated with the FLAG (G-CSF plus fludarabine and Ara-C) regimen. *Cytokine Mol Ther*. 1995;1:301-307.
16. Estey F, Thall P, Andreeff M, et al. Use of granulocyte colony-stimulating factor before, during, and after fludarabine plus cytarabine induction therapy of newly diagnosed acute myelogenous leukemia or myelodysplastic syndromes: comparison with fludarabine plus cytarabine without granulocyte colony-stimulating factor. *J Clin Oncol*. 1994;12:671-678.
17. Saito K, Nakamura Y, Aoyagi M, et al. Low-dose cytarabine and aclarubicin in combination with granulocyte colony-stimulating factor (CAG regimen) for previously treated patients with relapsed or primary resistant acute myelogenous leukemia (AML) and previously untreated elderly patients with AML, secondary AML, and refractory anemia with excess of blasts in transformation. *Int J Hematol*. 2000;71:238-244.
18. Lowenberg B, van Putten W, Theobald M, et al. Effect of priming with granulocyte colony-stimulating factor on the outcome of chemotherapy for acute myeloid leukemia. *N Engl J Med*. 2003;349:743-752.
19. Amadori S, Suciu S, Jehn U, et al. Use of glycosylated recombinant human G-CSF (lenograstim) during and/or after induction chemotherapy in patients 61 years of age and older with acute myeloid leukemia: final results of AML-13, a randomized phase-3 study. *Blood*. 2005;106:27-34.
20. Okamoto S, Takahashi S, Wakui M, et al. Treatment of advanced myelodysplastic syndrome with a regimen including recombinant human granulocyte colony-stimulating factor preceding allogeneic bone marrow transplantation. *Br J Haematol*. 1999;104:569-573.
21. Takahashi S, Oshima Y, Okamoto S-I, et al. Recombinant human granulocyte colony-stimulating factor (G-CSF) combined conditioning regimen for allogeneic bone marrow transplantation (BMT) in standard-risk myeloid leukemia. *Am J Hematol*. 1998;57:303-308.
22. Takahashi S, Iseki T, Ooi J, et al. Single-institute comparative analysis of unrelated bone marrow transplantation and cord blood transplantation for adult patients with hematologic malignancies. *Blood*. 2004;104:3813-3820.
23. Mori T, Aisa Y, Yokoyama A, et al. Total body irradiation and granulocyte colony-stimulating factor-combined high-dose cytarabine as a conditioning regimen in allogeneic hematopoietic stem cell transplantation for advanced myelodysplastic syndrome: a single-institute experience. *Bone Marrow Transplant*. 2007;39:217-221.
24. Sorror ML, Maris MB, Storer B, et al. Comparing morbidity and mortality of HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative and myeloablative conditioning: influence of pretransplantation comorbidities. *Blood*. 2004;104:961-968.
25. Diaconescu R, Flowers CR, Storer B, et al. Morbidity and mortality with nonmyeloablative compared with myeloablative conditioning before hematopoietic cell transplantation from HLA-matched related donors. *Blood*. 2004;104: 1550-1558.
26. Sorror ML, Maris MB, Storb R, et al. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. *Blood*. 2005;106: 2912-2919.

27. Przepiorka D, Weisdorf D, Martin P, et al. Consensus conference on acute GVHD grading. *Bone Marrow Transplant*. 1995; 15:825-828.
28. Shulman HM, Sullivan KM, Weiden PL, et al. Chronic graft-versus-host syndrome in man: a long-term clinicopathologic study of 20 Seattle patients. *Am J Med*. 1980;69:204-217.
29. Riddell S, Appelbaum FR, Buckner CD, et al. High-dose cytarabine and total body irradiation with or without cyclophosphamide as a preparative regimen for marrow transplantation for acute leukemia. *J Clin Oncol*. 1988;6:576-582.
30. Kamani N, Bayever E, August CS, Bunin N, Goldwein JW, D'Angio GJ. Fractionated toral-body irradiation preceding high-dose cytosine arabinoside as a preparative regimen for bone marrow transplantation in children with acute leukemia. *Med Pediatr Oncol*. 1995;25:179-184.
31. Imamura M, Asano S, Harada M, et al. Current status of hematopoietic cell transplantation for adult patients with hematologic diseases and solid tumors in Japan. *Int J Hematol*. 2006;83: 164-178.
32. Ferrant A, Labopin M, Frassoni F, et al. Karyotype in acute myeloblastic leukemia: prognostic significance for bone marrow transplantation in first remission: a European Group for Blood and Marrow Transplantation study. Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation (EBMT). *Blood*. 1997;90:2931-2938.
33. Suciu S, Mandelli F, de Witte T, et al. Allogeneic compared with autologous stem cell transplantation in the treatment of patients younger than 46 years with acute myeloid leukemia (AML) in first complete remission (CR1): an intention-to-treat analysis of the EORTC/GIMEMAAML-10 trial. *Blood*. 2003;102:1232-1240.
34. Gale RP, Horowitz MM, Weiner RS, et al. Impact of cytogenetic abnormalities on outcome of bone marrow transplants in acute myelogenous leukemia in first remission. *Bone Marrow Transplant*. 1995;16:203-208.